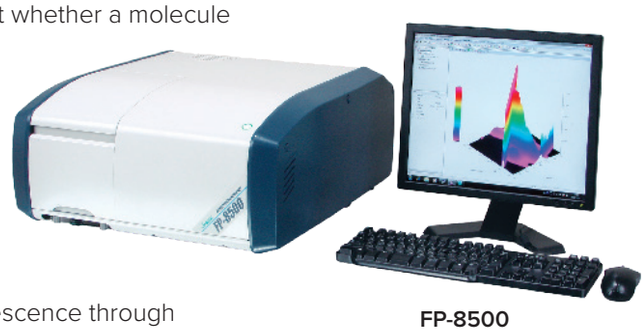
Application Note
FP-0008

Obtaining Absolute Quantum Yields Using an Integrating Sphere

Introduction

Different molecular and environmental conditions not only effect whether a molecule will fluoresce or not, but can also determine the intensity of the emitted fluorescence radiation. A molecule's efficiency to fluoresce is described by its quantum yield and is defined as the ratio of the number of photons absorbed to the number of photons emitted by a sample. There are two methods for measuring the fluorescence quantum yield: the absolute method and the relative method. The absolute method directly obtains the quantum yield by detecting all sample fluorescence through the use of an integrating sphere. The relative method compares the fluorescence intensity of a standard sample with the fluorescence intensity of an unknown sample to calculate the quantum yield of the unknown sample. Therefore, the obtained results depend on the accuracy of the standard sample's quantum yield value.



FP-8500
Spectrofluorometer

In this application note, the fluorescence spectra for several samples will be obtained using an integrating sphere and the quantum yields will be calculated using the absolute method and compared with literature values.

Keywords

FP-8500, ILF-835, FWQE-880, Integrating sphere, Fluorescence, Quantum yield, Absolute method

Experimental

Rhodamine B was used to correct the excitation spectrum prior to obtaining the absolute quantum yield calculations. The synchronous spectrum of a standard white diffuser plate was measured between 250 - 450 nm and the emission spectrum of a calibrated halogen light source was measured from 450 - 700 nm to correct the emission spectrum.

To measure the incident light using the integrating sphere, a spectrum is measured with nothing in the sample cell holder, as seen on the left in Figure 1. The sample is then placed into the integrating sphere and the spectrum is measured. The spectra of both the incident light and sample are shown in Figure 2. The incident photon (S_0) peak appears in the excitation spectrum, illustrated in blue while the excitation and emission peaks indicate the number of photons unabsorbed (S_1) and emitted (S_2) by the sample, respectively.

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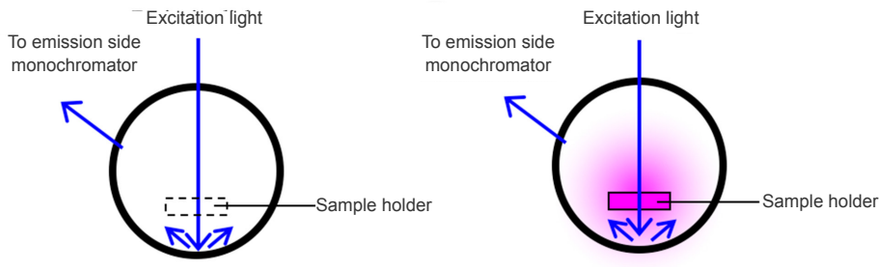

Figure 1. (Left) Setup for measuring the incident light and (right) sample using the integrating sphere.

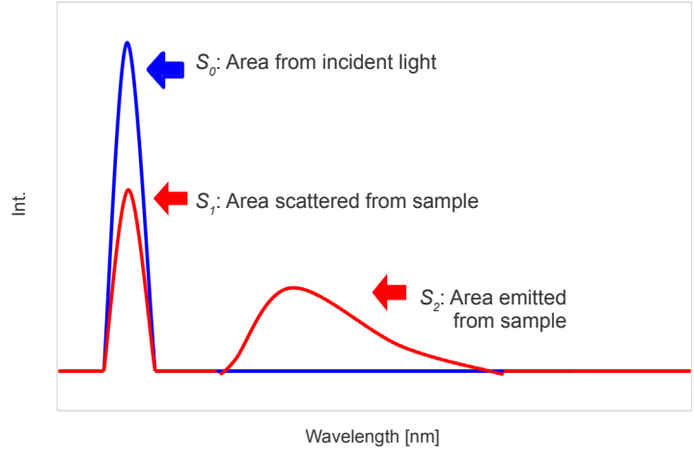

Figure 2. Excitation and emission spectra of incident (blue) and scattered (red) photons.

Measurement Conditions			
	Quinine Sulfate	**Fluorescin**	**Tryptophan**
Excitation Bandwidth	5 nm	5 nm	5 nm
Emission Bandwidth	5 nm	5 nm	5 nm
Excitation Wavelength	350 nm	475 nm	280 nm
Scanning Speed	200 nm/min	200 nm/min	200 nm/min
Data Interval	0.5 nm	0.5 nm	0.5 nm
Response Time	05. sec	05. sec	05. sec
PMT Voltage	350 V	250 V	400 V

200 ppm of quinine sulfate in 1.0 N H_2SO_4, 15 ppm of fluorescein in 0.1 N aqueous NaOH, and 200 mg/mL of tryptophan in ultra pure water were prepared.

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Results

The fluorescence spectra of quinine sulfate, fluorescein, and tryptophan are shown in Figures 3, 4, and 5, respectively.

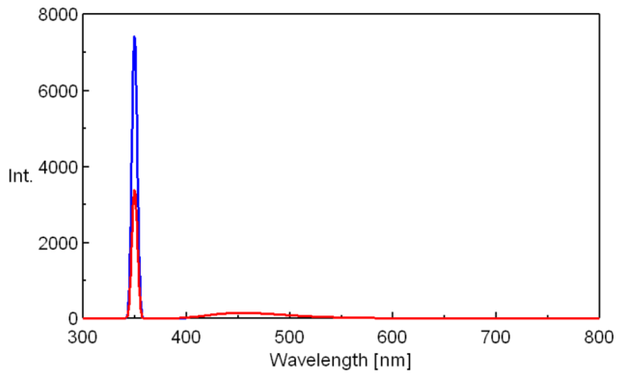

Figure 3. Emission spectra of the incident light (blue) and quinine sulfate (red).

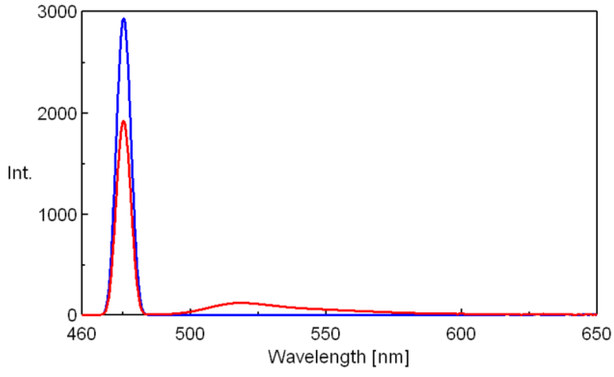

Figure 4. Emission spectra of the incident light (blue) and fluorescein (red).

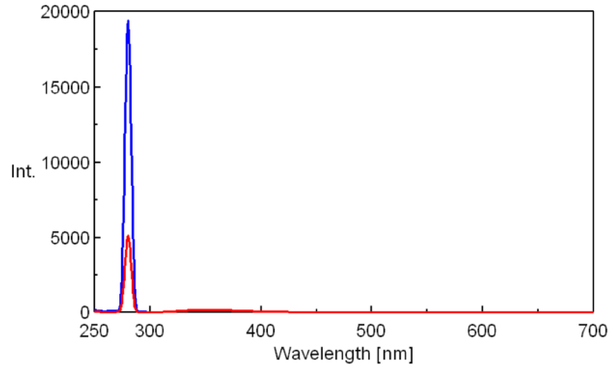

Figure 5. Emission spectra of the incident light (blue) and tryptophan (red).

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Table 1 provides the values for the area under the maxima in the spectra shown in Figures 3-5.

Table 1. Peak areas from the fluoresce spectra of the three sample solutions.

Sample Name	Area from Incident Light [S_0]	Area Scattered from Sample [S_1]	Area Emitted from Sample [S_2]	Scattered WL Range (nm)	Emitted WL Range (nm)
Quinine Sulfate	48267	22538	14304	320 - 365	365 - 750
Fluorescein	19174	12515	6116	465 - 485	485 - 630
Tryptophan	136135	35842	12101	270 - 290	290 - 550

The quantum yields for the three samples were calculated by the following equations:

$$\text{Sample Absorptance (\%)} = \frac{S_0 - S_1}{S_0} \times 100$$

$$\text{Internal Quantum Yield (\%)} = \frac{S_2}{S_0 - S_1} \times 100$$

$$\text{External Quantum Yield (\%)} = \frac{S_2}{S_0} \times 100$$

The calculated quantum yield results using the values in Table 1 are shown in Table 2.

Table 2. Calculated quantum yield results.

Sample Name	Sample Absorbance	Internal Quantum Yield	External Quantum Yield	Internal Quantum Yield Literature Values
Quinine Sulfate	53.3%	55.6%	29.6%	50 - 57%[1]
Fluorescein	34.7%	91.8%	31.9%	85 - 92%[1]
Tryptophan	73.7%	12.1%	8.9%	12 - 14%[2]

Conclusion

The quantum yields of quinine sulfate, fluorescein, and tryptophan have been calculated from the fluorescence spectra of the samples. The obtained results are within range of the published literature values.

References

1. The Spectroscopical Society of Japan, Japan Scientific Societies Press.
2. Principles of fluorescence spectroscopy, Joseph R. Lakowicz, Springer.

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